IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

HARDERN et al

Serial No. **09/508,195**

Filed: **March 8, 2000**

For: **NOVEL COMPOUNDS**

Atty. Ref.: **3764-2**

Group: **1624**

Examiner: **Ford, J.**

* *

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

DECLARATION

Sir:

We, Anthony H. Ingall and Brian Springthorpe, do hereby declare and state as follows:

1. Anthony H. Ingall:

My current job title with AstraZeneca UK Limited is "Associate Principal Scientist". I have been employed by AstraZeneca UK Limited and its predecessor companies for 26 years and, during all of that time, I have worked in the Department of Medicinal Chemistry primarily as a laboratory worker and supervisor of laboratory workers, but I also have certain administrative duties. At the time of the work in question, I directed a team of 5 people. I received my undergraduate degree from Imperial College, University of London, England in 1970 and my PhD also from Imperial College in 1973. My technical expertise is in the field of medicinal chemistry and synthetic organic chemistry.

2. Brian Springthorpe:

My position within AstraZeneca UK Limited is "Team Leader Medicinal Chemistry, AZ Charnwood". I have been employed with AstraZeneca UK Limited and

its predecessor companies for 30 years. I currently have responsibility for 6 people. I obtained a B.Sc. Chemistry (Hons 1st class) in 1976 from De Montfort University, and an M.Sc. in 1978 from the University of East Anglia. I have in excess of 20 years experience in the fields of medicinal chemistry and synthetic organic chemistry.

3. Attached are copies of laboratory note book pages of research chemists working under our direct supervision and control on this project. For the nine compounds exemplified in the application, five of the compounds were synthesized prior to September 21, 1998. The details are as follows:

Example Number	AR-C Number	Chemist Name	Lab Notebook Number	Page Numbers
1	130284	Andrew Bailey	2307	159-160
2	126583	Gemma Cansell	2345	25-26
3	126532	Simon Gulle	2335	47-48
4	130234	Barry Teobald	2295	178-183
5	130237	Barrie Martin	2274	157-156

4. In each case, attached is a copy of the cover showing the book number together with the relevant pages from the book. In several cases, the chemist lists the experiments carried out in a particular book at the front of the book. Where this is the case, such pages are attached to demonstrate that this represents the standard notebook. Where this was not available, the next page in the book is attached as evidence of routine standard use of the book.

5. The practice involves dating the top of the page at the start of the experimental with the date an experiment is started, demonstrating conception of the idea. Upon completing the experiment, the chemist signs and dates the end of the experimental write-up. The book is then countersigned and dated (note that for book 2274 page 158, there is a typographical error on the sign-off date - signed as "97" not "98").

We each declare that all statements herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Anthony H. Ingall

19 November 2001
Date

Brian Springthorpe

19 November 2001
Date

Attachments

2307

Andrew Bailey

2242 ⟵ 2307 ⟶ 2407

previous book

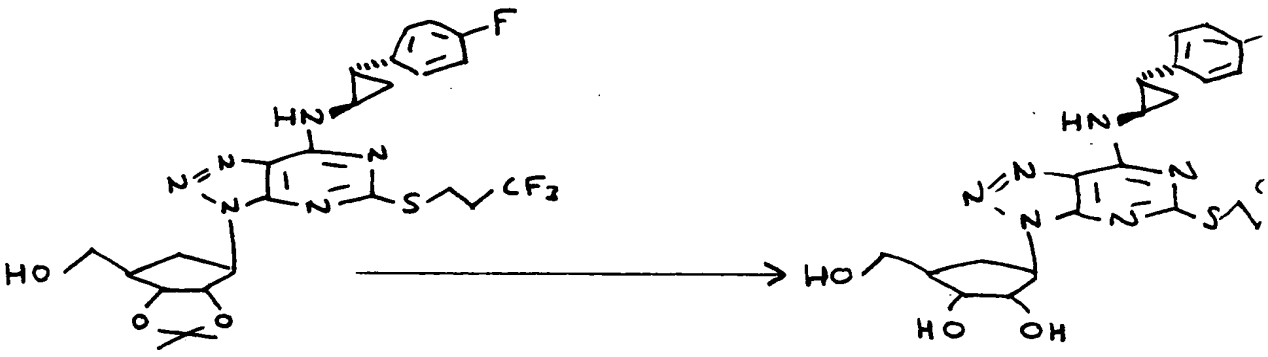
next book

1: 13/8/98, A. Bailey

Prep^n of [1R-(1a,2a,3b,5b(1R*,2S*))]-3-[7-((2-(4-fluorophenyl)cyclopropyl)amino)-5-(3,3,3 trifluoropropylthio)-3H-1,2,3-triazolo[4,5-d] pyrimidin-3-yl)-5-hydroxymethyl-cyclopentane-1,2-d

AB

AB



AB

$C_{25}H_{28}F_4N_6O_3S$ (568.5) $C_{22}H_{24}F_4N_6O_3S$ (528

AB

Method

AB

The S.M (1.4g) was dissolved in a mixture of trifluoroacetic acid (10ml) and water (2ml) and the soln left to stand for 50 mins at R.T

AB

TLC EtOAc/isohex (1/1)

SM.	O	
R.M	O	O O

AB

work up RM diluted with EtOAc and washed with xs aq bicarb, organic layer dried, filtered and vacc'd down

AB

purification Flash column. 5 → 6% MeOH in $CHCl_3$

AB

Yield = 440 mg 'pure' + 250 mg 'less pure'

AB

AN° 298797 of the 440 mg of 'pure' foam

AB

HPLC 99.4%. major impurity 0.23%.

AB

MS APCI (+ve), M+H = 529

AB

NMR δ_{DMSO} 9.42 (d, 1H, NH), 7.27-7.22 (m, 2H, aroms), 7.14-7.08 (m, 2H, aroms), 5.01-4.95 (m, 2H, CH+OH), 4.73-4.70 (m, 2H, 2OH), 4.44-4.41 (m, 1H, CH), 3.87-3.84 (m, 1H, CH), 3.50-3.45 (m, 2H, CH_2), 3.26-3.13 (m, 3H, 3CH), 2.60-2.55 (m, 1H, CH), 2.28-2.20 (m, 2H, CH_2), 2.10-2.06 (m, 1H, CH), 1.90-1.80 (m, 1H, CH), 1.49-1.46 (m, 1H, CH), 1.33-1.30 (m, 1H, CH) missing H under solvent peak at 2.5? 5

AB

MA Theory is for 0.42 moles H_2O ie MWt = 536

Theory	C = 49.30	H = 4.67	N = 15.68	S = 5.98
found	48.91	4.38	15.62	5.92

AB

410 mg submitted as 130284XX

AB

AN° 298852 of the less pure 250 mg

AB

MS/NMR okay, HPLC 97.6%. major imp 0.48%.

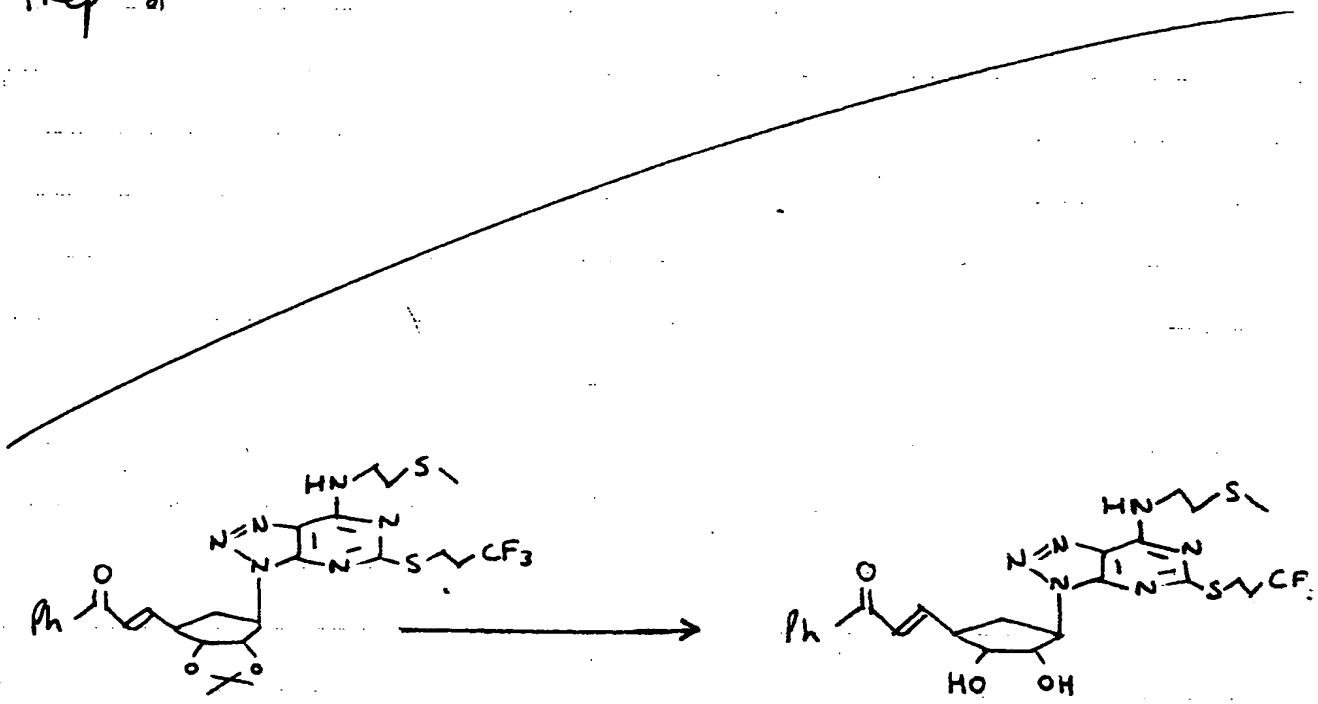
AB

180 mg as 130284XX Batch 2

COMPLETED

A. Bailey 22/9/98 READ AND UNDERSTOOD BY R. Jewell 02 OCT 1998

17/8/98 Prep^ of
A. Bailey



AD

$C_{27}H_{31}F_3N_6O_3S_2$ (608·7) $C_{24}H_{27}F_3N_6O_3S$ (568·6)

AB

Method

AB

The S.M (0·3g) was dissolved in a mixture of trifluoroacetic acid (10ml) and water (2ml) and the R.M was left to stand for 30 mins at R.T.

AB

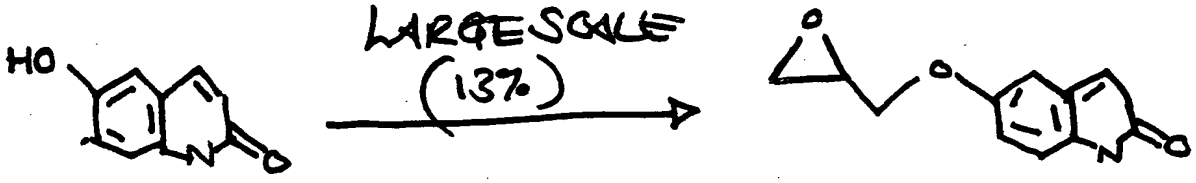
TLC 4% MeOH in $CHCl_3$

SM ·		0
RM ·	00	·

AB

work up RM partitioned between EtOAc and xs aq bicarb, the organic layer was dried, filtered and vacced down.

21



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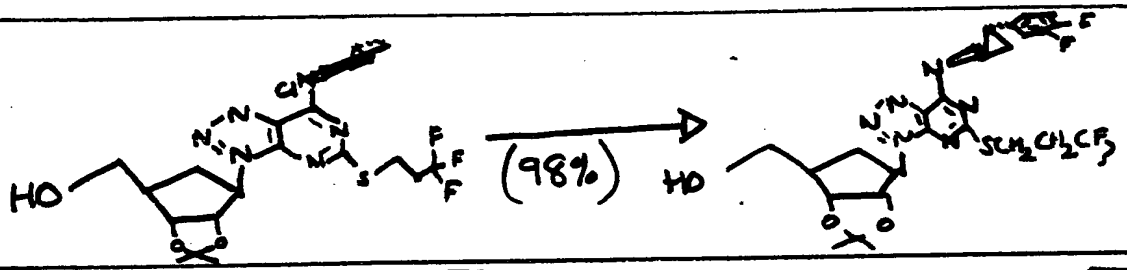

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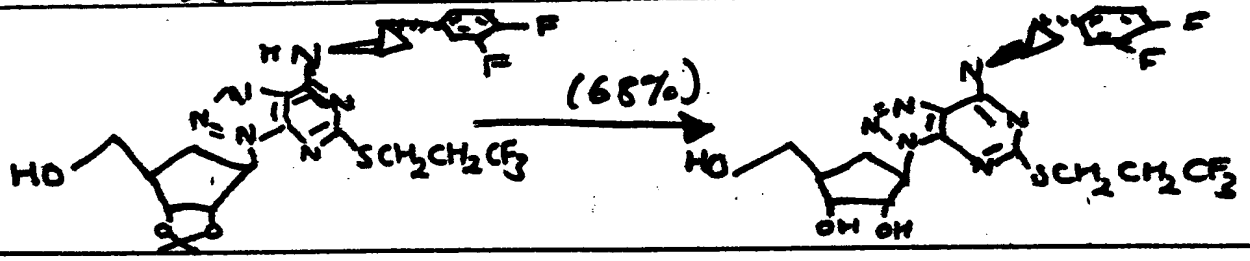

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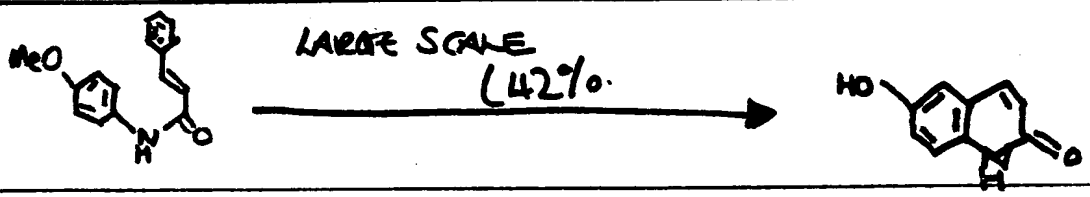

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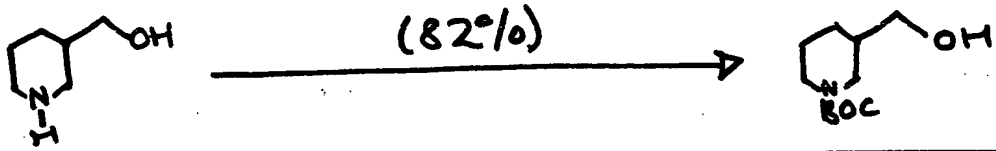

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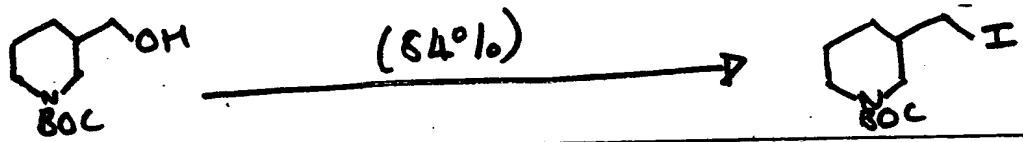

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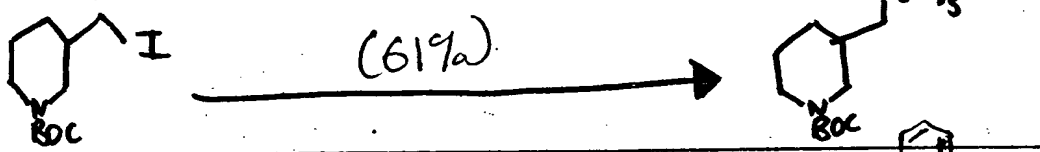

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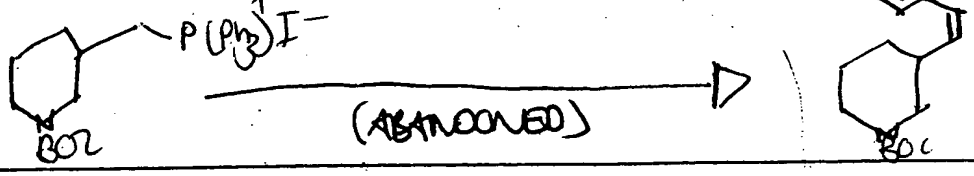

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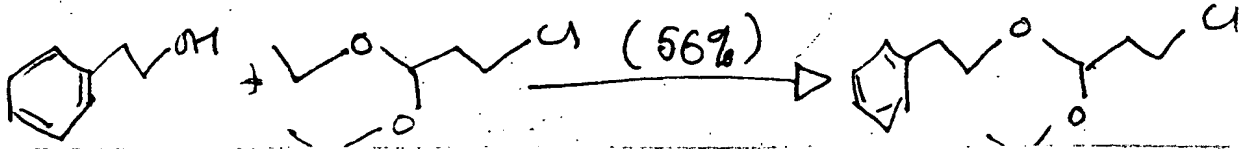
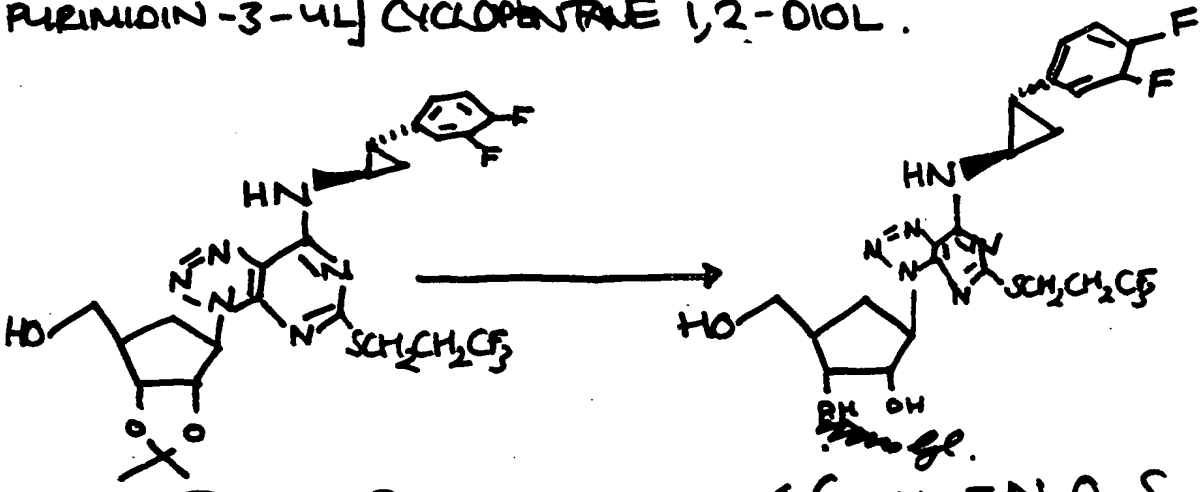


9 27/7/98. PREPARATION OF [3aR-(3aα,4α,6α(1R*,2S*),(6aα)]-6-(7-[[2-(3,4-DIFLUOROPHENYL)CYCLOPROPYL]AMINO]-5-(3,3,3-TRIFLUOROPROPYLTHIO)-3H-[1,2,3]-TRIAZOLO[4,5-d] PYRIMIDIN-3-YL] CYCLOPENTANE 1,2-DIOL.



$C_{25}H_{27}F_5N_6O_3S$ (586.584)

$C_{22}H_{23}F_5N_6O_3S$ (546.519)

METHOD The S.M (360mg, 0.614mmol) was dissolved in 10ml of methanol. The solution was treated with dilute aq. HCl (2ml, 3M). The RM was left to stand at RT for 2½ hours

TLC EtOAc/Isohexane (-partn)

SM.		O
MK.	O	O
RM.	O	(::

WORK UP R.M was partitioned between EtOAc + $NaHCO_3$. Organic layer was separated, dried and Vacod. down.

PURIFICATION. FLASH COLUMN, BIOTAGE, EthylAcetate (3) Isohexane (1). Removes S.M and final Product.

YIELD 229mg of White foam (68%).

AN° APCI(+VE) M+H = 547.3 / (-VE) = 545.2.

MS 298022

NMR δDMSO D_6, 9.43 (O,1H,NH), 7.35–7.28 (M,2H,ARO) 7.02-7.14 (M,1H,ARO), 5.01 - 4.96 (M,2H), 4.72-4.69 (T,2H) 4.44 - 4.41 (Q,1H) 3.87 - 3.84, (Q,1H) 3.50-3.44 (M,2H) 3.25 - 3.12 (M,3H), 2.58 - 2.56 (M,1H), 2.28-2.21 (M,3H), 1.76-1.91 (M, 1H), 1.52-1.50 (M,1H), 1.39 - 1.37 (M, 1H), 1H MISSING, SUSPECTED UNDER SOLVENT.

10/9/98

HPLC 99.7%

COMPLETED J. McNally
READ AND UNDERSTOOD BY 18/9/98

ELEMENTAL Theory C=48.35% H=4.24% N=15.38% S=5.87% F=17.38%
FOUND 49.38 4.71 15.26 5.71
(1% extra on hydrate)

IR

cm-1	%T
3288	96.03
1520	75.
1454	75.71
1435	76.17
1366	87.35
1322	87.14
1274	85.43
1240	72.47
1132	76.35
1087	73.28
1042	72.06
991	74.84
955	84.16
891	78.89
812	83.72
772	82.47
688	76.17
638	79.12
	73.92

120mgs entered as:-
135mgs

126583 XX

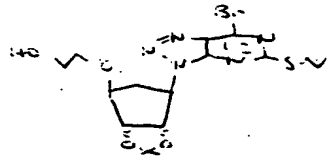
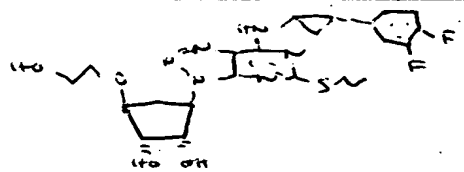
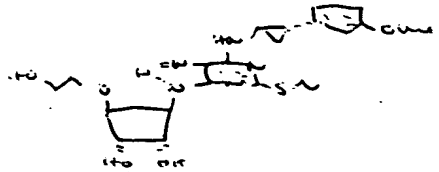
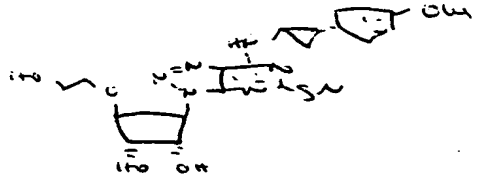
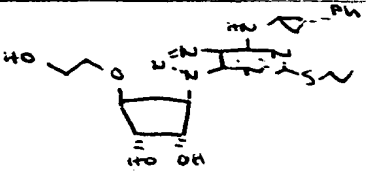
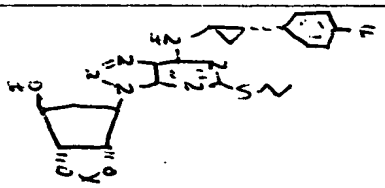
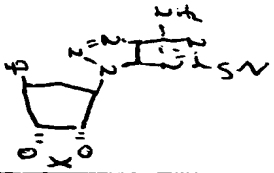
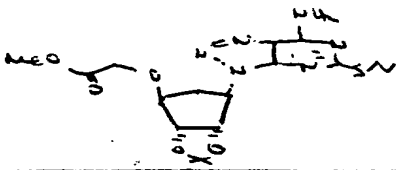
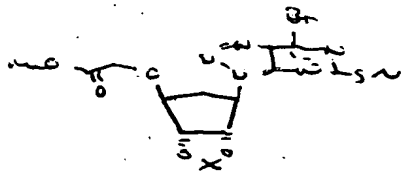
Simon D Guile

Medicinal Chemistry

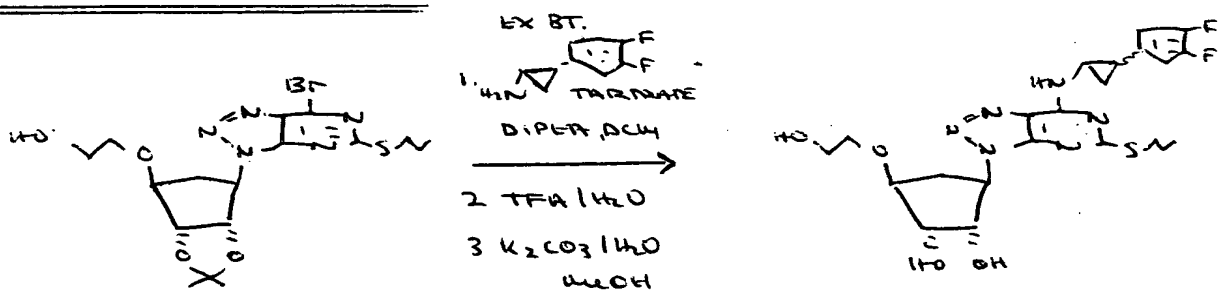
Astra Charnwood

Previous Book 2250

Next Book 2509

PLATE	PREPARATION OF...	YIELD	AR#
45.		42%	—
47.		48%	AR-C 126532XX
49.		73%	AR-C 126533XX
51.		53%	AR-C 126534XX
53.		31%	AR-C 120492XX BATCH 3
55.		82%	—
57.		100%	—
59.		38%	—
61.		42%	—

15/7/98 PREPARATION OF ~~[2-OH]~~ [1R-[1α,2β,3β(1R*,2S*),5β]]-3-[7-[(3,4-DIFLUOROPHENYL)CYCLOPROPYL]AMINO-5-(PROPYLTHIO)-3H-1,2,3-TRIAZOLO[4,5-d]PYRIMIDIN-3-YL]-5-[(2-HYDROXY)ETHOXY]CYCLOPENTANE-1,2-DIOL



(474.383) (522.675)

2335/45A $C_{23}H_{28}F_2N_6O_4S$

AMOUNTS

(474.383)	SUBSTRATE	75 mg,	0.16 mmol, 1eq
(319.26)	AMINE TARTRATE	66 mg,	0.21 mmol, 1.3eq
0.742 (129.25)	DIPEA	83 µl,	0.47 mmol, 3eq
	DCM	5 ml	

EXPERIMENTAL

A mixture of the above reagents was stirred at rt for 24 hrs. The reaction mixture was absorbed on to silica and purified (Biotage 1/1 EtOAc/Hex).

⇒ 2335/47A

AN 297523

LC/MS APCI+ 563 $(M+H)^+$ >99% PURE

The protected compound was treated with TFA/H2O (10 ml; 9:1) for 10 mins then concn in vacuo. The residue (mix of prod + TFA ester) was treated with K_2CO_3 (100 mg) in MeOH/H2O (10 ml; 1:1) for 1 hr.

This mixture was concentrated to remove MeOH. The remainder was partitioned between water (20 ml) and EtOAc (3×20 ml). The combined organic phase was dried ($MgSO_4$) and concentrated then triturated with pentane to produce a solid

⇒ 2335/48A Purified RPHPLC ⇒ 2335/48B 40mg, 48%

AN 297547 (48A) / 297735 (48B)

LC/MS 98.4% MAJOR IMPURITY 1.6% APCI+ 523 ($M+H^+$)

IR

EA FOUND C 50.64% H 5.43% N 15.87% S 5.72%

REQUIRED C 51.10% H 5.59% N 15.55% S 5.93%

FOR $C_{23}H_{28}F_2N_6O_4S \cdot H_2O$ FW 540.59.

^1H NMR DMSO 0.79–1.00 (m, 3H), 1.20–1.75 (m, 4H), 1.96–2.30 (m, 2H), 2.58–2.70 (m, 1H), 2.80–3.20 (m, 3H), 3.43–3.58 (m, 4H), 3.73–3.80 (m, 1H), 3.90–3.96 (m, 1H), 4.50–4.61 (m, 2H), 4.96 (q, J = 9.0 Hz, 1H), 5.05 (d, J = 3.9 Hz, 1H), 5.11 (d, J = 6.3 Hz, 1H), 7.00–7.10 (m, 1H), 7.22–7.40 (m, 2H), 9.36 and 8.97 (m, 1H)

30 mg Submitted as ~~AR~~

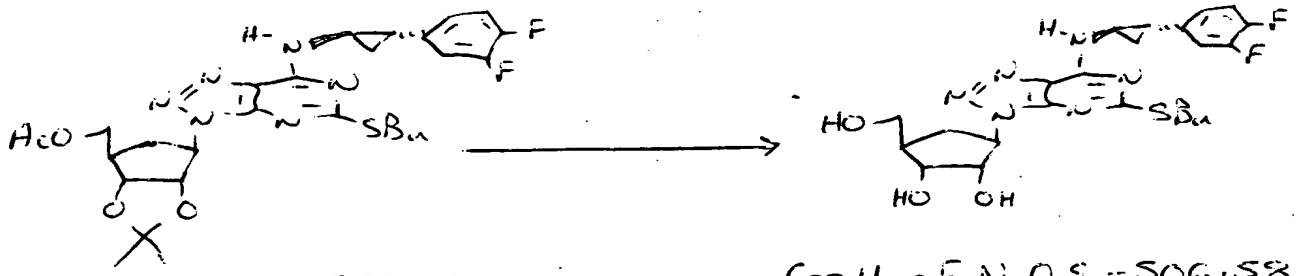
AR-C126532XX

COMPLETED 23/7/98 IAShalles 13/11/98

READ AND UNDERSTOOD BY

2295

29-7-98 The Attempted Preparation of [1S-(1α, 2α, 3β, 5β (1S*, 2R*))]-3-[5-Butylthio-7-[[2-(3,4-difluoro-phenyl)cyclopropyl]amino]-3H-[1,2,3]-triazolo[4,5-d] pyrimidin-3-yl]-3-hydroxymethyl-cyclopentane-1,2-diol

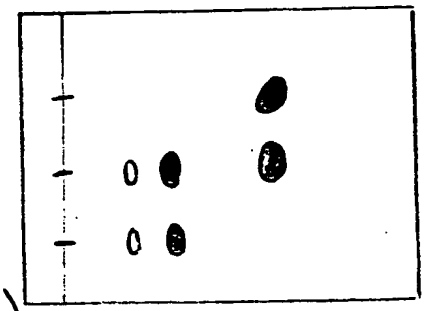


$C_{28}H_{34}F_2N_6O_4S = 588.68$ $C_{23}H_{28}F_2N_6O_3S = 506.58$

Protected nucleoside	0.226g	0.384mmol 1eqiv 2295/175/2
80% HCO_2H/H_2O	10ml	
10% K_2CO_3/H_2O	1ml	
MeOH	10ml	

A colourless solution of [3aR-(3aα, 4α, 6α(1R*, 2S*), 6aα)]-acetic acid, [[6-[5-butylthio-7-[[2-(3,4-difluoro-phenyl)cyclopropyl]amino]-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-3-yl]-tetrahydro-2,2-dimethyl-4H-cyclopenta-1,3-dioxol-4-yl]methyl]ester (0.226g, 0.384mmol) in 80% acetic acid/water (10ml) was heated in an oil bath at 80° for 1hour. Tlc indicated that some reaction had taken place:-

B.T.



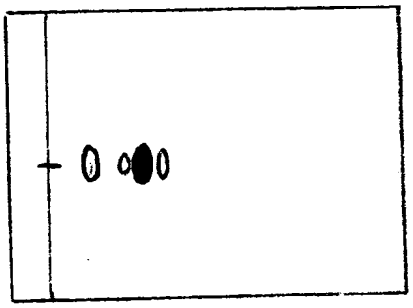
2295/175/2
Mixed Spot
Reaction Mixture
($NaHCO_3/H_2O$/EtOAc)

SiO_2
5% MeOH/CH_2Cl_2
U.V.

B. Teobald. 12-8-98

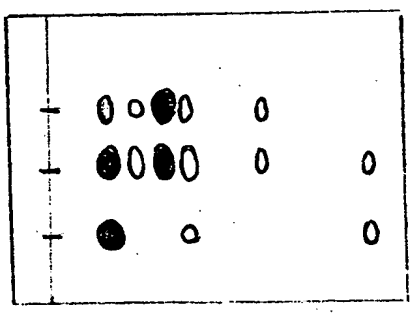
The reaction mixture was allowed to cool and was cautiously poured into saturated sodium bicarbonate solution (150ml). The resulting emulsion was extracted with ethyl acetate (3x 35ml). The combined organic phases were washed with saturated sodium bicarbonate solution (70ml), dried ($MgSO_4$) and concentrated in-vacuo to give a pale yellow gum (0.220g, 2295/178/1). Tlc indicated that 2295/178/1 was a mixture :-

B.T.



B.T. 2295/178/1 (0.220g) was dissolved in methanol (10ml) and to this ~~colourless~~ pale yellow solution was added a 10% aqueous solution of potassium carbonate (1ml). The resulting pale yellow solution was stirred at room temperature for 1/2 hour. Tlc indicated that some reaction had taken place :-

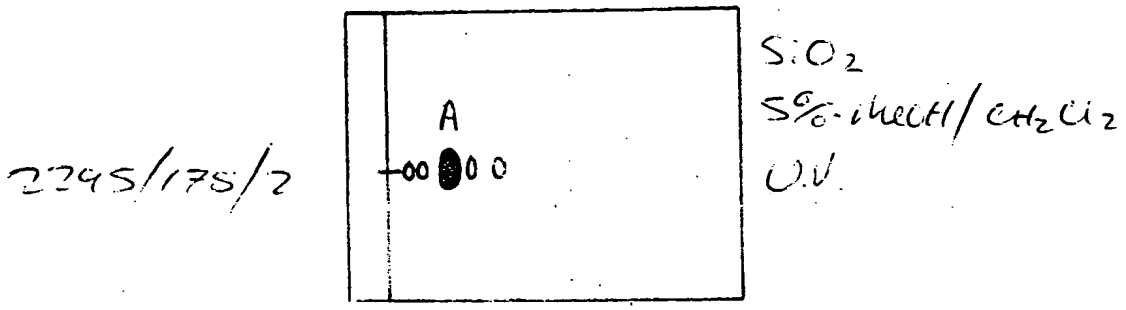
B.T.



The reaction mixture was neutralized to pH = 7

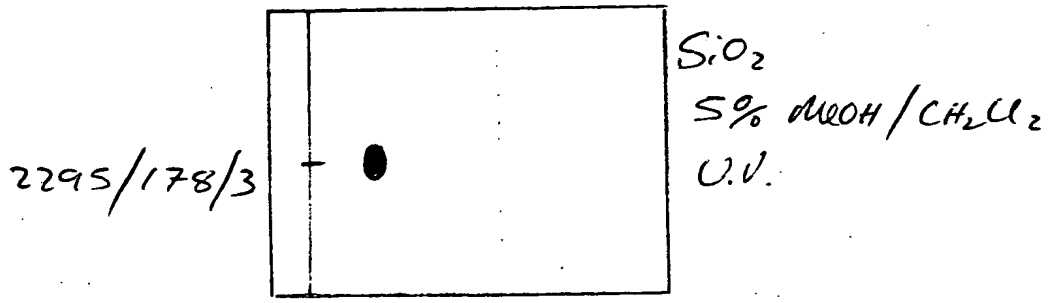
using a few drops of acetic acid and was then concentrated in-vacuo to give a sticky off-white residue (0.543g, 2295/178/2). Tlc indicated that 2295/178/2 was a mixture :-

B.T.



2295/178/2 (0.543g) was dissolved in a mixture of dichloromethane and methanol and was adsorbed onto flash silica (5ml, FISHER Matrex 60, 35-70μm) in-vacuo. The resulting free-flowing white powder was loaded onto a column of silica (54ml, as above) and eluted with 5% methanol in dichloromethane. Fractions containing essentially pure component 'A' were combined and concentrated in-vacuo to give a colourless residue which was dissolved in diethyl ether and re-concentrated to give a white foam (0.166g, 85%, 2295/178/3). Tlc indicated that 2295/178/3 was pure :-

B.T.



2295/178/3 (0.166g) was dissolved in a mixture of tetrahydrofuran and acetonitrile

B. Teobald. 12-8-98

to a concentration of approximately 20 mg/ml. The resulting solution was filtered and aliquot containing ~ 20 mg were purified by preparative HPLC on a Waters Novapak column eluted with 0·1% aqueous ammonium acetate and acetonitrile, isocratic mixture, 50% acetonitrile over 15 minutes, monitoring at 254 nm. Fractions containing the main peak were combined and concentrated in-vacuo to remove most of the acetonitrile from the mixture. The resulting sticky suspension was freeze dried to give a white fluffy solid (0·70 g, 2295/178/4).

AN298282 2295/178/4

NMR

^1H D_6-DMSO Shows the material to be the desired product, essentially pure:-

B.T.

δ_H 9·34 & 8·94 (Total 1H, 2 x bd, NH); 7·32 (2H, m, H-12 & H-15); 7·06 (1H, m, H-16); 4·99 (2H, m, H-1' & 1 x OH); 4·72 (2H, m, 2 x OH); 4·43 (1H, m, H-2'); 3·88 (1H, m, H-3'); 3·79 & 3·16 (Total 1H, 2 x m, H-8); 3·48 (2H, m, H-6'); 3·10 & 2·93 (Total 2H, 2 x m, H-17); 2·26 (1H, m, 1 x H-5'); 2·11 (2H, m, H-4' & H-10); 1·84 (1H, m, 1 x H-5');

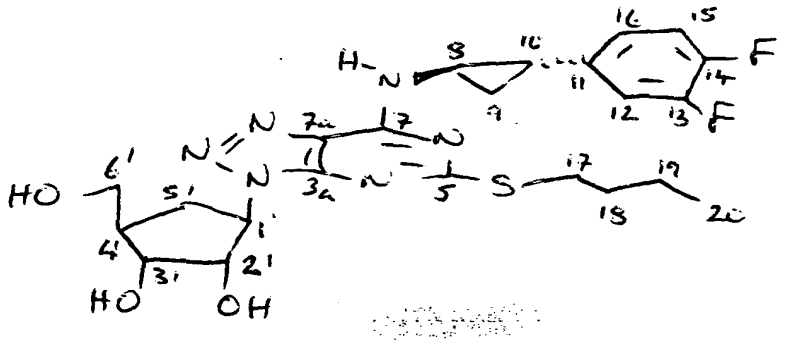
B.T. 1·65 & 1·46 (Total 2H, 2 x m, H-18); 1·55 (1H, m, 1 x H-9); 1·37 (1H, m, 1 x H-9); 1·24 (2H, m, H-19), 0·91 & 0·81 (Total 3H, 2 x t, 7Hz & 7·3Hz, H-20)

B.T.

^{13}C D_6-DMSO shows the material to be the desired product, essentially pure:-

δ_C 169.1 (C-5); 153.9 (C-7); 149.4 (dd, 245 Hz & 12 Hz, C-13); 149.3 (C-3a); 147.8 (dd, 243 Hz & 13 Hz, C-14); 139.2 (C-11); 123.2 (C-7a); 122.8 (C-16); 117.0 (d, 17 Hz, C-15); 114.8 (d, 18 Hz, C-12); 74.9 (C-2'); 71.8 (C-3'); 63.0 (C-6'); 62.2 (C-1'); 45.4 (C-4'); 34.0 (C-8); 31.0 (C-17); 30.1 (C-18); 29.0 (C-5'); 24.0 (C-10); 21.2 (C-19); 15.0 (C-9); 13.5 (C-20)

B.T.



B.T.

IR. Okay:-

Wave Number (cm-1)	Threshold (%T)
2703	100.2
2361	99.36
2340	99.89
1609	84.73
1589	86.21
1520	85.94
1454	92.2
1430	93.59
1322	81.81
1275	87.42
1211	89.07
1115	89.71
1044	89.71
992	92.51
892	92.97
857	94.53
808	91.41
773	86.68
617	85.54
579	84.26

Teobald.

B.T.

HPLC Symmetry C8 0.1% NH_4OAc (aq) / CH_3CN 25-95% CH_3CN

RT (mins)	%
2.18	99.7

B. Teobald. 12-8-98

MS LC/APCI(+ve) 507 $(M+H)^{\oplus}$
507 (100%)

B.T.

m.p. No melting point as material is a freeze dried solid.

B.T.

Elem.

	%C	H	N	S
Found	53.41	5.47	16.00	6.26
	53.58	5.67	16.30	6.22

• $1/2 H_2O$ Requires
⇒ MW = 515.57

B.T.

78mg Submitted as AR-C130234XX
B. Teobald. 12-8-98

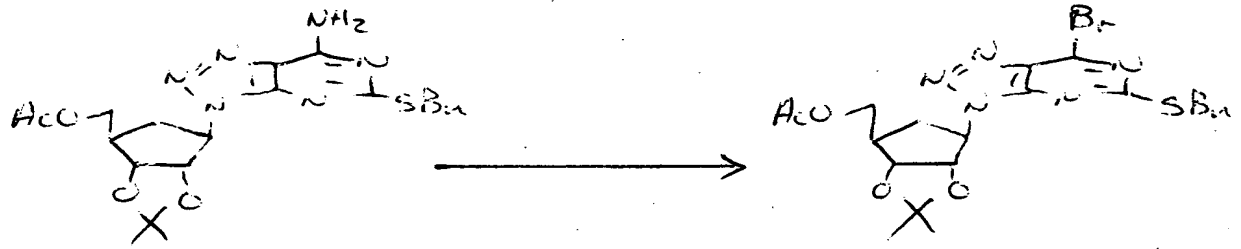
A

COMPLETED —
READ AND UNDERSTOOD BY S.C.K. 9-9-98

30-7-98 The Attempted Preparation of [3aR-(3ax, 4x, 6x, 6ax)]-Acetic acid, [[6-[7-bromo-5-butylthio-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-tetrahydro-2,2-dimethyl-4H-cyclopenta-1,3-dioxol-4-yl]methyl] ester

Ref: 2295/172

B.T.

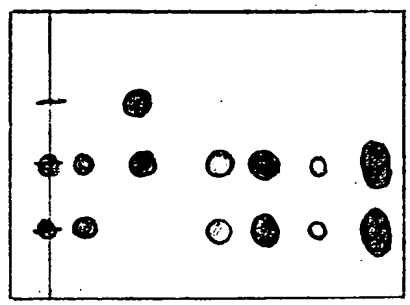


$C_{19}H_{28}N_6O_4S = 436.54$ $C_{19}H_{26}BrN_5O_4S = 500.42$

Protected nucleoside	2.13g	4.88 mmol	2295/164/2
Bromoform	23ml		
ONO	4.7ml		

B.T. A yellow solution of [3aR-(3ax, 4x, 6x, 6ax)]-acetic acid, [[6-[7-amino-5-butylthio-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]tetrahydro-2,2-dimethyl-4H-cyclopenta-1,3-dioxol-4-yl]methyl] ester (2.13g, 4.88 mmol) in bromoform (23ml) and isoamyl-nitrite (4.7ml) was heated in an oil bath at 80° for 1/2 hour. Tlc of the resulting golden yellow solution indicated that the reaction was complete:-

B.T.



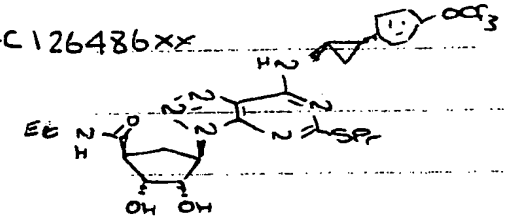
2295/164/2
Mixed Spot
Reaction Mixture

SiO_2
60% Et_2O/Isohexane
U.V.

B. Teobald. 12-8-98

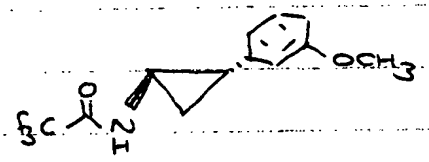
2274

AR-C126486XX

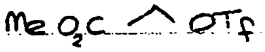


58%

149.

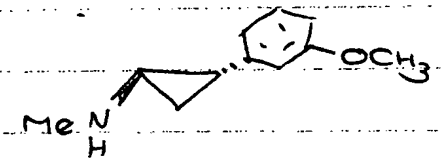


93%

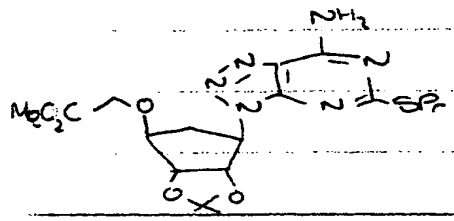


83%

150.

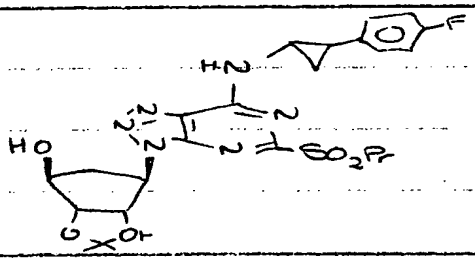


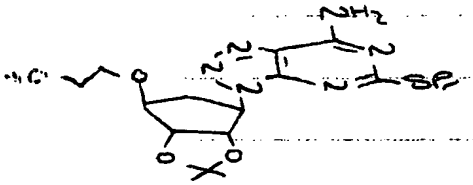
151.



41%

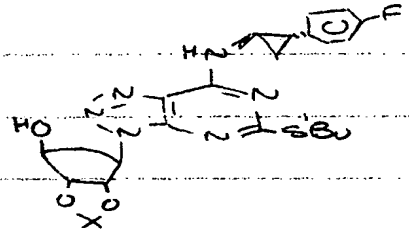
153.



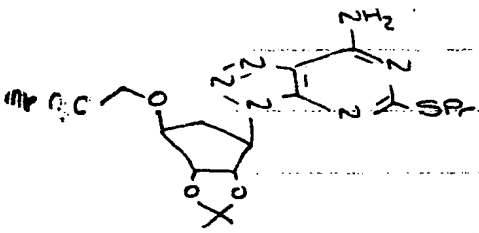


66%

155.

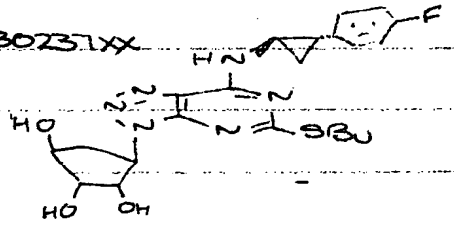


84

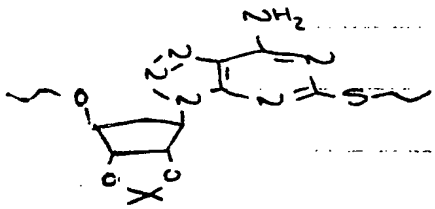


45%

157. AR-C130237XX

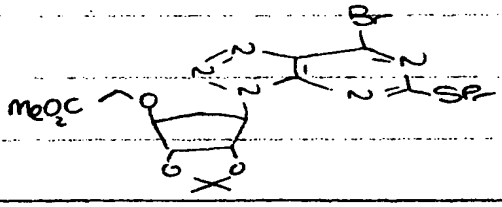


95%

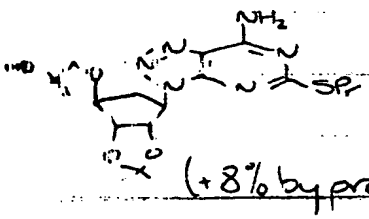


74%

159.

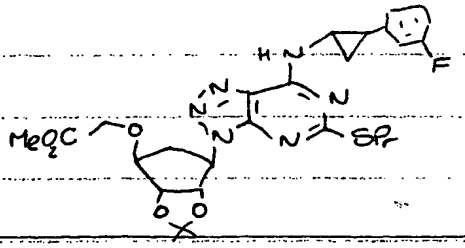


52%



(+8% by product) 65%

161.

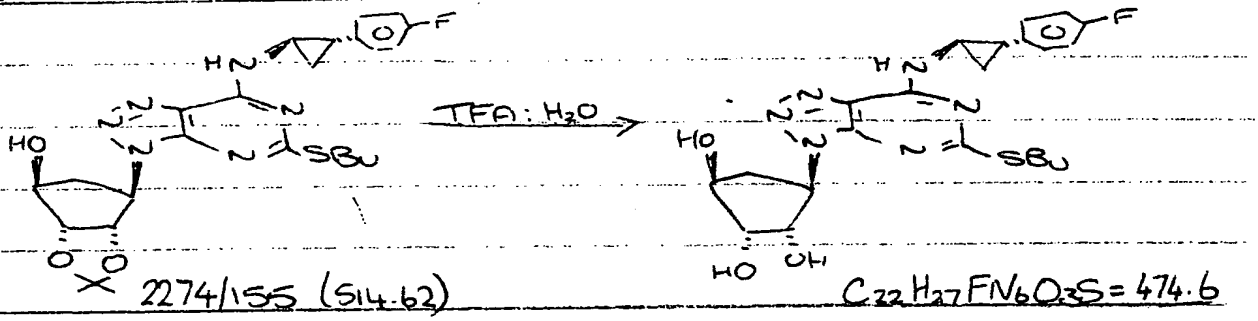


75%

AR-C 130237 XX

30-7-98

1S-[1α,2β,3β,4α(1S*,2R*)]-4-[7-(2-(4-Fluorophenyl)cyclopropylamino-5-propylthio-3H-1,2,3-triazolo[4,5-d]pyrimidin-3-yl]cyclopentane-1,2,3-triol.



2274/155 (514.62) $C_{22}H_{27}FN_6O_3S = 474.6$

2274/155 (690mg, 1.34mmol) was dissolved in water (10ml) and TFA (10ml), after stirring for 1hour reaction was complete by HPLC.

The reaction mixture was added dropwise to sat. $NaHCO_3$ (250ml) soln, and then extracted into ethylacetate (3×100ml). The organics were dried ($MgSO_4$), filtered and concentrated to dryness, the residue was then purified by RPHPLC to give a white solid (620mg, AN 298217)

Yield = 620mg (95%)

AN 298217

Infra-red: 1321, 1611, 1588, 1511, 1044, 1228, 818, 789, 1277, 1189, 1098, 834

HPLC: A7505AP.M RT = 2.14min, 99.55%

mass spec: APCI +ve M+H = 475.1 (100%)

Elem. anal $0.8H_2O = 489.01$	C	H	N	S
$C_{22}H_{27}FN_6O_3S$	53.99	5.85	17.18	6.54
Found	54.04	5.82	17.02	6.54

AN 298217

proton NMR (300 mH_3, d6-DMSO)

0.80 t, J = 7.5 H_3, 3H 1)

1.22 sex, J = 7.2 H_3, 2H 2)

1.30-1.35m 1H 7a)

1.41-1.53m 3H 7b) 3)

1.86-1.91m 1H 8)

2.11-2.15m 1H 18a)

2.51-2.59m 1H 18b)

2.80-3.00m 2H 4)

3.13-3.35m 1H 6)

3.77 bs 1H 16)

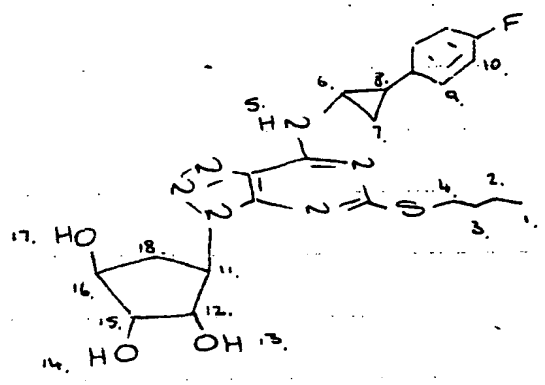
3.93 bs 1H 15)

4.63-4.67m 1H 12)

4.90-4.99m 1H 11)

7.11 t, J = 9.0 H_3, 2H, 9)

7.22-7.26m 2H 10)



melting point: 75-78°C.

590 mg submitted as AR-C130237xx

6-8-97